Industry allocated project number	PHI allocated project number
PPRI13/16	

AGRICULTURAL RESEARCH COUNCIL
PLANT PROTECTION RESEARCH
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FINAL REPORT

PPRI 13/16

Determine the transmission dynamics of plants infected with mixed GLRaV-3 variants

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FINAL REPORT
2016

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1. PROGRAMME AND PROJECT LEADER INFORMATION

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2. PROJECT INFORMATION

Research Organisation Project number	PPRI13/16
Project title	Determine the transmission dynamics of plants infected with mixed GLRaV-3 variants
Short title	Transmission dynamics of plants with mixed variant infections

Fruit kind(s)	grapevine		
Start date (mm/yyyy)	01/04/2014	**End date** (mm/yyyy)	31/03/2016

Key words	Mixed GLRaV-3 variant infections, transmission, *P. ficus*

Approved by Research Organisation Programme leader (tick box) | X |

3. EXECUTIVE SUMMARY

Previously, an extensive survey revealed the dominant occurrence of GLRaV-3 variants II and VI in South African vineyards. Further investigations, that included variant-vector interactions, followed. In this study the transmission dynamics of mixed variant infections in selected source plants were determined. The overall objective of the project was to assist in identifying any biological and transmission differences among the various variants of GLRaV-3. A transmission experiment using *Planococcus ficus* was carried out using source plants that were infected with seven different combinations of GLRaV-3 variants. The selected plants were co-infected with other grapevine-infecting viruses. A RT-PCR HRM analysis was done to determine the transmission efficiency of individual GLRaV-3 variants from the mixed variant infections in source plants. Detection of the presence of other grapevine infecting viruses, i.e. *Grapevine virus A* (GVA)*, Grapevine virus B* (GVB) and *Grapevine rupestris stem pitting virus* (GRSPaV), was carried out using virus-specific primers. The study demonstrated that GLRaV-3 variants from groups I and II established better in recipient plants during transmission either as single transmission or in combination with each other. The exact interaction between variants in a plant is still unknown but it is clear that competition between variants exists, either in the mealybug vector or in the recipient plant after transmission. GVA was transmitted in higher frequencies than GVE. The interaction between GVA and the group II variant needs to be investigated in more detail.

Impact on industry:
Not all grapevine farmers have the financial means to remove and replace infected plants or blocks and therefore the problem of leafroll will continue to pose a serious economic problem in certain grape-producing regions. This study aimed to support the management strategies of the industry through gaining knowledge of GLRaV-3 variants and their transmission that will help to solve future management problems, for instance when resistance breeding may be considered in future.

4. PROBLEM IDENTIFICATION AND OBJECTIVES

The transmission dynamics of mixed variant infections in local vineyards play a key role in understanding the biological aspects of leafroll spread, on which there is limited data available presently.

Results from a recent survey study showed that GLRaV-3 variants of groups II and VI occurred predominantly in vineyards locally (refer to Winetech funded project GenUS11-1). This is an indication that these two variants of the virus might be more easily transmitted by the mealybug vectors, or that a combination of these variants in a plant can cause a more aggressive spread of the virus. The transmission dynamics of variants I and VI occurring in the Napa Valley in the USA were examined by Blaisdell et al. (2012). The authors concluded that vector transmission of the group VI variant alone was more frequent, followed by transmission with mixed infections of the two, while transmission with the group I variant alone was the least common. This was the first evidence that GLRaV-3 variants are biologically distinct.

The overall objective of the project was to assist in identifying any biological and transmission differences among the various variants of GLRaV-3, if they exist.

The overall objective was supported by 1) a transmission experiment with *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), the most abundant mealybug vector in South African vineyards, as a vector and source plants infected with seven different combinations of GLRaV-3 variants (Table 1); and 2) utilising a RT-PCR HRM analysis to determine the transmission efficiency of individual GLRaV-3 variants from the mixed variant infections in source plants. Detection of the presence of other grapevine infecting viruses was included.

5. WORKPLAN (MATERIALS AND METHODS)

A transmission experiment was performed during April-June 2014. Seven source plants were selected infected with different combinations of GLRaV-3 variants (Table 1). Other grapevine-infecting viruses were also present in the plants. Mealybug transmissions were carried out at the University of Pretoria (UP) with *P. ficus*. Duplicate mealybug colonies were kept on butternut (*Juglans cinerea*) at UP and ARC-PPR. Leaves from infected source plants listed in Table 1 were placed in cages with mealybug nymphs to allow the first-instar nymphs (crawlers) to walk onto the infected leaves. After an acquisition access period (AAP) of 24 hours was applied, after which, 20 crawlers were transferred to healthy *Vitis vinifera* cv Cabernet franc recipient plants using leaf cages. The inoculation access period (IAP) was set at 24 hours. The leaf cages were removed and plants treated with insecticide (Confidor). Treated plants were transferred to facilities at ARC-PPR where they were monitored.

Table 1. Virus populations in source plants

Plant number	Cultivar	GLRaV-3 variant	Other viruses
26/22/18	Chardonnay	I, VI, VII	GVA, GVE, GRSPaV
8/7/1	Cabernet sauvignon	II, III	GVA
3/17/16	Cabernet sauvignon	II, VI, VII	-
14/12/4	Cabernet franc	I, III	GVE
20/7/2	Cape Riesling	I, II, VI, VII	GVA, GVE
10/20/6	Shiraz	I, II	-
13/2/19	Cabernet sauvignon	II, VI	GRSPaV

The viral population in each plant was determined with specific RT-PCR tests to confirm the presence of other grapevine-infecting viruses, i.e. GVA, GVE, GRSPaV in the source plants.

6. RESULTS AND DISCUSSION

In the previous study (PPRI11/18), where the transmission efficiency of four variants was tested, the four GLRaV-3 variants, representing groups I, II, III and VI, in single infected vines, or when occurring in combination with GVA, were transmitted equally well under controlled conditions. No indication of variant-vector specificity was detected. The *Vitivirus*, GVA, was transmitted at higher frequencies from GLRaV-3/GVA infected plants and transmitted without the simultaneous transmission of GLRaV-3.

In this study, 155 mealybug transmissions were carried out with 20 first-instar nymphs per recipient plant. The GLRaV-3 variant status of recipient plants was determined using a one-step real-time RT-PCR HRM assay. The virus status of other grapevine-infecting viruses was determined using virus-specific RT-PCR assays. The success rate of the transmissions was above 80% for five of the source plants (Figure 1), and this is considerably higher than the 9 to 18% success rate obtained in the previous experiment with single mealybug transmissions.

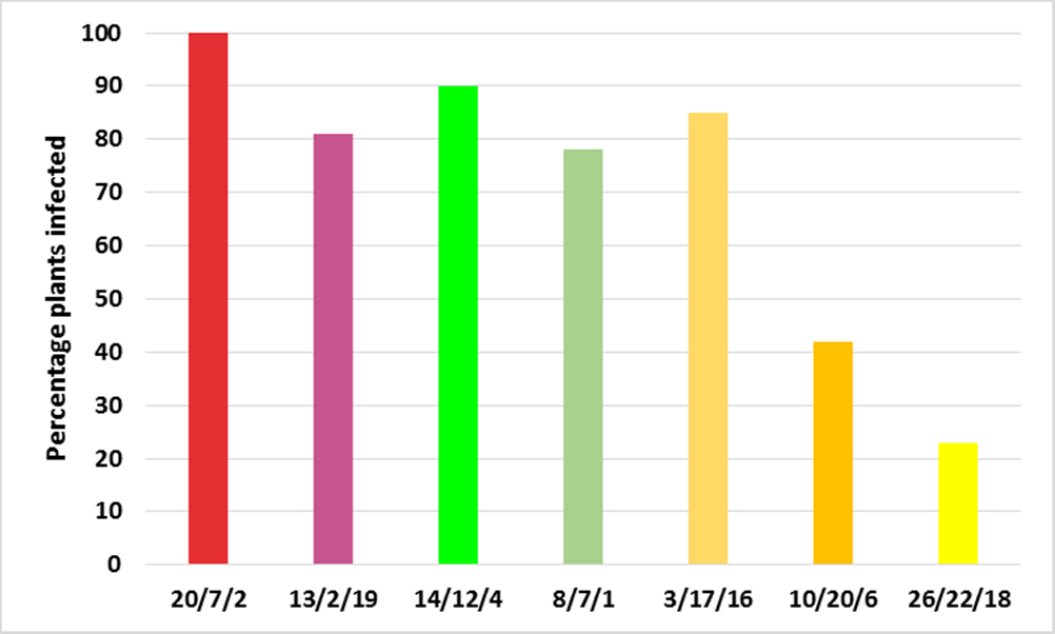

Figure 1. Percentage plants infected from selected source plants

Transmission of GLRaV-3 variants:
RNA extracted from recipient plants was analysed using the one-step real-time RT-PCR HRM assay and the following results were obtained.

20/7/2: The source plant was infected with variants I, II and VI; the group II variant was transmitted at a higher frequency as well as the combination of group I + II variants (Fig 2A)
13/2/19: The source plant was infected with group II and VI variants; again the group II variant was transmitted more efficiently (Fig 2B)
14/12/4: The source plant was infected with group I and III variants; the group I variant was slightly more readily transmitted, but the combination of variants I+III was transmitted most efficiently (Fig 2C)
8/7/1: The source plant was infected with group II and III variants; the group II variant was transmitted more efficiently than group III variants (Fig 2D)
3/17/16: The source plant was infected with group II and VI variants; the group II variant was transmitted most efficiently (Fig 2E)
10/20/6: The source plant was infected with group I and II variants; the combination of group I+II variants was transmitted slightly more efficiently (Fig 2F)
26/22/18: The source plant was infected with group I and VI variants; the group I variant was transmitted most efficiently (Fig 2G)

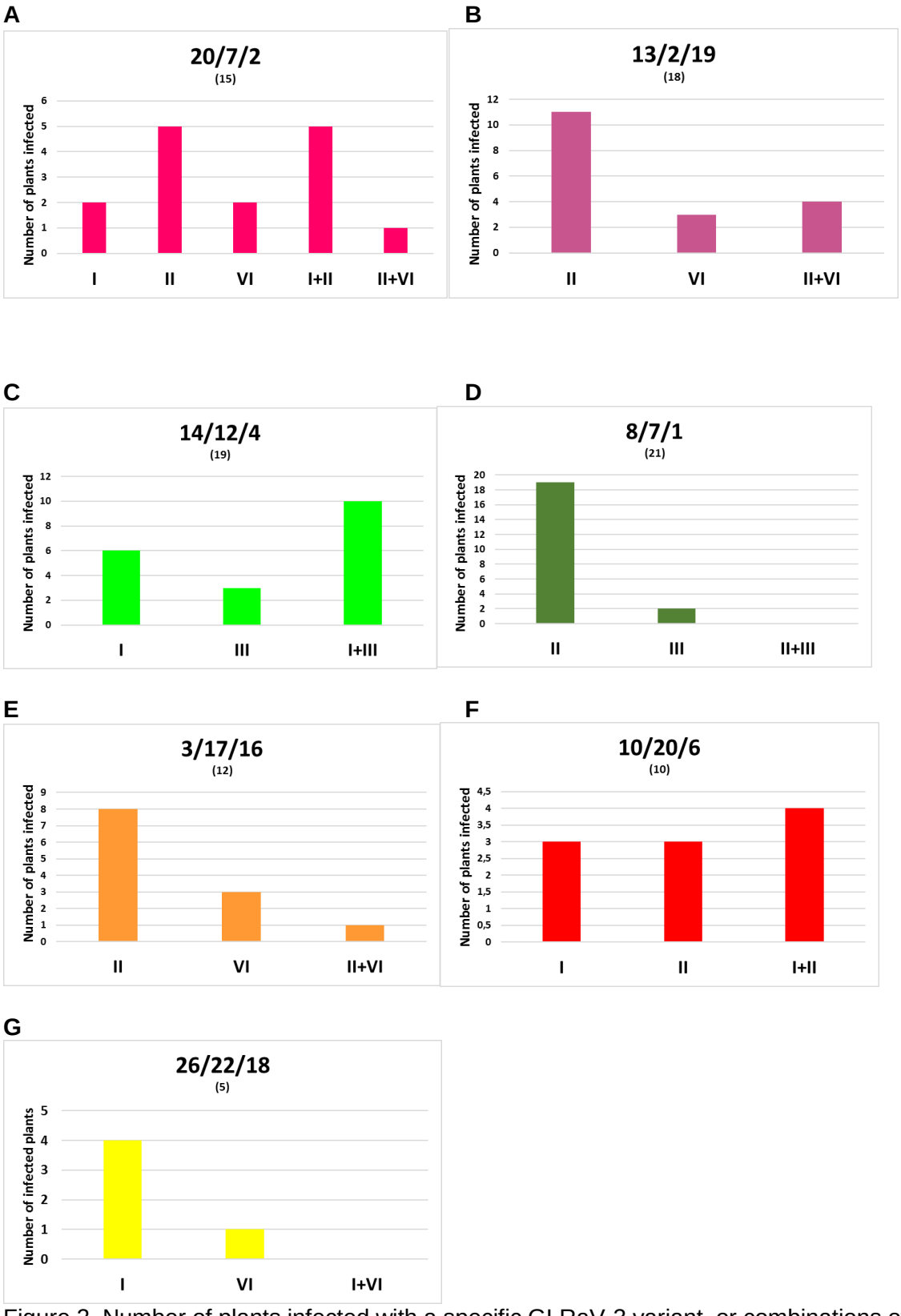

Figure 2. Number of plants infected with a specific GLRaV-3 variant, or combinations of variants, in the transmission experiment from selected source plants

The transmission results indicate that variants I and II established better in recipient plants either as single transmission or in combination with each other than the other variants tested. The group VI variant was not transmitted as effectively as the group I and II variants. A reduced establishment of one variant in mixed transmissions was observed, possibly due to competition

(Figure 2A, B, D, E, G). In two of the source plants (Figure 2 C, F), the combination of variants was transmitted more effectively. The specific interactions between variants within the vector and the recipient plant is still unclear and needs to be investigated further.

Transmission of other grapevine-infecting viruses:
Virus-specific RT-PCR's were done to detect the transmission frequency of GVA, GVE and GRSPaV in recipient plants.

Table 2. Percentage plants infected with other grapevine infecting viruses, *i.e.* GVA, GVE and GRSPaV

	GLRaV-3 variants	Other viruses	Transmission results		
			GVA	**GVE**	**GRSPaV**
26/22/18	I,VI, VII	GVA, GVE, GRSPaV	0%	18%	0%
8/7/1	II, III	GVA	22%	-	-
20/7/2	I, II, VI, VII	GVA, GVE	60%	0%	-
13/2/19	II, VI	GRSPaV	-	-	5%
14/12/4	I, III	GVE	-	0%	-

The results in Table 2 do not allow for clear cut conclusions on the transmission of other grapevine-infecting viruses. A high transmission rate of GVA was recorded with source plant 20/7/2, but GVE was not transmitted from this plant. In source plant 8/7/1, GVA was transmitted to 22% of the recipient plants. It is interesting to note that the GLRaV-3 variant from group II was present in both these plants, 8/7/1 and 20/7/2. We could speculate that GVA might be transmitted more effectively in the presence of the group II variant, but this hypothesis needs to be investigated in more detail. In source plant 26/22/18, GVE was transmitted but not GVA, and the group II variant was absent. Further investigations are needed.

7. COMPLETE THE FOLLOWING TABLE

Milestone	Target Date	Extension Date	Date completed	Achievement
1. Mealybug transmissions were done from each source plant infected with different combinations of GLRaV-3 variants to 15-20 recipient cv. Carbernet franc plants each using a standardised transmission protocol	April/May 2014		April-June 2014	155 mealybug transmissions were done with 20 mealybugs per recipient plant
2. RNA was extracted from recipient plants 8 months after transmissions. RNA was used as template in a one-step real-time RT-PCR HRM assay to determine how four GLRaV-3 variants were	February-April 2015		March-May 2015	The assay was used to determine the GLRaV-3 variant status in recipient plants.

transmitted from mixed infected plants				
3. Presented partial results at ICVG, Ankara, Turkey	September 2015		September 2015	An oral presentation was given at the international conference
4. Journal publication(s) – final milestone	March 2017		ongoing	Transmission results from projects PPRI11/18 and PPRI13/16 will be combined in a paper

8. CONCLUSIONS

The results from this study demonstrated that GLRaV-3 variants from groups I and II established better in recipient plants during transmission either as single transmission or in combination with each other. The exact interaction between variants in a plant is still unknown but it is clear that competition between variants exists, either in the mealybug vector or in the recipient plant after transmission. The group VI variant was not transmitted at the same rate as the group II variant and even in combination with each other. Other grapevine-infecting viruses were detected in recipient plants. GVA was transmitted in higher frequencies than GVE. The interaction between GVA and the group II variant needs to be investigated in more detail.

9. ACCUMULATED OUTPUTS

1. 155 mealybug transmissions were done from seven selected source plants containing different combinations of GLRaV-3 variants and other grapevine infecting viruses. Twenty mealybugs per recipient plant were used. (2014)
2. A RT-PCR HRM assay was used to determine which GLRaV-3 variants were transmitted successfully. Virus-specific tests were used to determine the virus status of other grapevine-infecting viruses. (2015)
3. An oral presentation was given at the ICVG international conference in Turkey, 2015

a) TECHNOLOGY DEVELOPED, PRODUCTS AND PATENTS

None- a previously developed technology, RT-PCR HRM assay, was used in the analysis of recipient plants

b) SUGGESTIONS FOR TECHNOLOGY TRANSFER

A scientific publication will be submitted as well as a popular article in Winelands. The article will include results from projects PPRI11/18 and PPRI 13/16

c) HUMAN RESOURCES DEVELOPMENT/TRAINING

None

d) PUBLICATIONS (POPULAR, PRESS RELEASES, SEMI-SCIENTIFIC, SCIENTIFIC)

Winelands article- in preparation
Scientific publications- in preparation

e) PRESENTATIONS/PAPERS DELIVERED

Jooste AEC, Krüger, K. (2015). Mealybug transmission efficiency of four Grapevine leafroll-associated virus 3 (GLRaV-3) genetic variants. 18th Congress of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine (ICVG), Ankara, Turkey, September 2015.

10. BUDGET

a) TOTAL COST SUMMARY OF THE PROJECT

YEAR	CFPA	DFTS	Deciduous	SATI	Winetech	THRIP	OTHER	TOTAL
2014					108000		35000	143000
2015					118000		40000	158000

b) FINAL BUDGET/FINANCIALS OF PROJECT

Project duration	Proposed budget	Actual cost incurred	Variance	Notes
TOTAL INCOME				
Industry Funding	226 000	226 000		
PHI Funding				
Other Funding	75 000	75 000		Glasshouse (PPRI) Insect facilities (UP)
TOTAL EXPENDITURE	301 000	301 000		
Running Expenses				
General operating costs (printing, communication, etc.)	126 000	126 000		All operating costs were spent (enzymes, laboratory consumables, pesticides for glasshouse)
Local Travel	20 000	10 000	10 000	Variance will be used to attend September 2016 feedback meeting
Running expenses SUB-TOTAL	146 000	146 000		All costs spent as indicated
HR Administration and Project Management				
HR Technical				
HR Research	80 000	80 000		Personnel costs over 2 years
Student Bursaries				
HR SUB-TOTAL	80 000	80 000		
OTHER EXPENSES				

Project duration	Proposed budget	Actual cost incurred	Variance	Notes
SURPLUS / DEFICIT	none			All funds allocated were spent as listed above

EVALUATION BY INDUSTRY

This section is for office use only

Project number	

Project name	

Name of Sub-Committee*	

Comments on project

Committee's recommendation

- Accepted. ☐
- Accepted provisionally if the sub-committee's comments are also addressed. Resubmit this final report by_____ ☐
- Unacceptable. Must resubmit final report. ☐

Chairperson_____ Date_____

*SUB-COMMITTEES

Winetech
Viticulture: Cultivation; Soil Science; Plant Biotechnology; Plant Protection; Plant Improvement;
Oenology: Vinification Technology; Bottling, Packaging and Distribution; Environmental Impact; Brandy and Distilling; Microbiology

Deciduous Fruit
Technical Advisory Committees: Post-Harvest; Crop Production; Crop Protection; Technology Transfer
Peer Work Groups: Post-Harvest; Horticulture; Soil Science; Breeding and Evaluation; Pathology; Entomology